(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 000 661 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
17.05.2000 Bulletin 2000/20

(51) Int. Cl.[7]: B01L 3/00, B01L 7/00

(21) Application number: 98120187.4

(22) Date of filing: 29.10.1998

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

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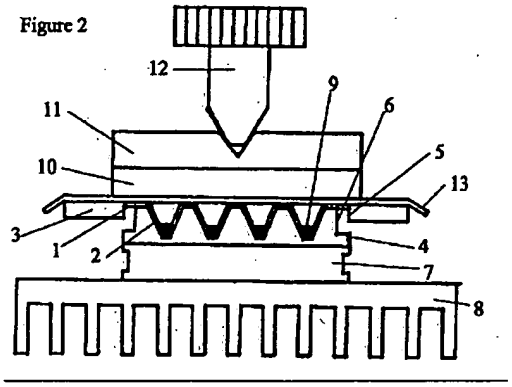
(54) Ultrathin-walled multiwell plate for heat block thermocycling

(57) Ultrathin-walled multiwell reactors for heat block thermocycling of samples comprising an array of small-volume wells of identical height with similarly shaped sample wells formed in the top surface of the heat block of the thermocycler are provided. The multiwell plates are preferentially vacuumformed out of a 30-50 micron thick thermoplastic film and can be used for rapid, oil-free temperature cycling of small (1-10 µl) volume samples.

Figure 2



EP 1 000 661 A1

Description

The invention relates to plastic plates for conventional heat block thermocycling of biological samples, particularly to multiwell plates. More specifically, it relates to ultrathin-walled multiwell plates with an improved heat transfer to small-volume samples. Such plates can be used for rapid temperature cycling of multiple, small-volume samples (i.e. 1-10 µl) by using heat block thermocyclers with an increased block temperature ramping (i.e. 4° C/second and greater) and standard heated-lid technology for sealing the plates.

Temperature cycling of biological samples is a central moment in DNA amplification by the polymerase chain reaction (PCR) (Saiki et al., Science, 239, 487-491 [1988]). Much effort is being expended in developing various alternative reactors and technologies for rapid temperature cycling of small-volume samples (Kopp et al., Science 280, 1046-1048 [1998]; Belgrader et al., J.Forensic Science 43, 315-319 [1998]; Swerdlow et al. Analytical Chem., 69, 848-855 [1997]; Wittwer et al., Analytical Biochem., 186, 328-331 [1990]; Wittwer and Garling, BioTechniques, 10, 76-83, [1991]; Woolley et al., Analytical Chem., 68, 4081-4086 [(1996]). However, these rapid DNA amplification technologies are connected with various disadvantages. For example, in these techniques the samples are handled and sealed one-by-one, sometime in a relatively cumbersome manner due to the special features of the microreactors. The delivering and recovering of the small samples is more complicated compared with conventional plastic tubes or microplates. In addition, the price of such reactors, as disposable PCR containers, is very high when compared to the conventional plastic tubes. The experimental throughput using the above systems is limited. Therefore, it is surprising that only little research has been conducted to improve the basic performance in sample size and speed of the widely used, conventional heat block thermocycling of samples contained in plastic tubes or multiwell plates. One known improvement of heat block temperature cycling of samples contained in plastic tubes has been described by Half et al. (Biotechniques, 10, 106-112, [1991] and U.S. Patent No 5,475, 610). They describe a special PCR reaction-compatible one-piece plastic microcentrifuge type tube, i.e. thin-walled PCR tube. The tube has a cylindrically shaped upper wall section and a relatively thin (i.e approximately 0.3 mm) conically-shaped lower wall section. The samples as small as 20 µl are placed in the tubes, the tubes are closed by deformable, gas-tight caps and positioned into similarly shaped conical wells machined in the body of the heat block. The heated cover compresses each cap and forces each tube down firmly into its own well. The heated platen (i.e. heated lid) serves several goals by supplying the appropriate pressure to the caps of the tubes: it maintains the conically shaped walls in close thermal contact with the body of the block; it prevents the opening of the caps by increased air pressure arising in the tubes at elevated temperatures. In addition, it maintains the parts of the tubes that project above the top surface of the block at 95° -100° C in order to prevent water condensation and sample loss in the course of thermocycling. This made it possible to exclude the placing of mineral oil or glycerol into the wells of the block in order to improve the heat transfer to the tubes and the overlaying of the samples by mineral oil that prevented evaporation but also served as added thermal mass. In addition, the PCR tubes can be put in a two-piece holder (US patent 5,710,381) of an 8x12, 96-well microplate format, which can be used to support the high sample throughput needs with any number between 1 and 96 individual reaction tubes. However, the thickness of the tube and the minimal sample volume are yet too high to enable rapid heat transfer. Therefore, the temperature equilibrium between the large sample and the block is reached relatively slow. Usually, the sample temperature lags behind the temperature of the heat block of the thermal cycler for 20-40 seconds and the total DNA amplification time of 1.5 hours or more is typical (see Wittwer and Garling, BioTechniques, 10, 76-83, [1991]). As the tubes are designed to hold relatively large sample volumes (50-100 µl) rather than small ones (0.5-10 µl), the use of small sample volumes in said tubes results in an increased vapor sorption due to the increased free internal surface of the tube. The multiwell thin-walled PCR plates have been commercially introduced as cost effective alternatives to the above mentioned trays of individual tubes. Such modern plates comprise arrays of conically-shaped wells (0.3mm) molded as a single plastic unit. They are manufactured in various formats to meet the needs of particular biomedical tasks (i.e. 24-well to 48-well plates for routine applications and 96-well to 384-well plates for large scale applications, respectively; see also below). Principally, the same heated-lid technology is used for sitting the arrayed conically shaped wells into the similarly tapered and arrayed wells machined in the body of the heating block. The only difference compared to the tube arrays concerns the sealing of the sample containers. The one-piece plates containing the samples are transferred to the sample block of the thermal cycler, upon sealing all the wells of the plates by a single silicon mat or a special sealing film (e.g. US Patent No 5,721,136). The major advantage of multiwell-PCR plates as one-piece plastic units is that they do not give any problems in handling of the small reactors arrayed in one unit in the course of injection molding of the plate when compared to individual PCR tubes or tubes arrayed in various holders (e.g. see Perkin Elmer Users Manuals, Part No. 0993-8660, 1992). This advantage has been realized in the high sample density 384-well PCR plates. Their well-volume is 40 µl with an inter-well distance of 4.5 mm. However, the aim of the 384-well plates concerns rather the increase of their sample-number capacity than the advantage of the small well-volume which could be taken to improve the sample size and the cycle speed. As the thickness, geometry and material (polypropylene) of the multiwell-PCR plates is the same as used for the above described tubes, both, the efficiency of the heat transfer and the minimal sample size are directly comparable. Although the modern heat block thermocyclers are capable to change the block temperature at a rate which is theoretically sufficient to perform an exponential amplification in less than 20 minutes (i.e. Primus-96; ramping rate 5° C/second; supplier: MWG-Biotech, Munich, Germany), the heat transfer from the block to the relatively large sample

volumes contained in the modern plastic PCR tubes or multiwell PCR plates is inefficient to reach these amplification times. Therefore, such PCR plates cannot be used for parallel rapid thermocycling of multiple samples. High troughput PCR can be achieved by either increasing the number of samples per run, e. g. 384-well plates and/or by reducing the amplification time. The latter has the advantage of a reduced turnaround, a very important aspect in, for example, rapid and cost effective PCR screening of pooled samples when serial rounds of PCR reactions and product analysis have to be performed.

The present invention concerns plastic multiwell plates for performing conventional heat block thermocycling of multiple samples. More specifically, it concerns ultrathin-walled multiwell plates for heat block temperature cycling of samples with a wall thickness of not more than 50 microns. Ultrathin-walled multiwell plates are suited for rapid, oil-free, heat block temperature cycling of small-volume samples (i.e. approximately 0.5-10 µl) whereas the lower limit is given by the reliability of the conventional pipetting systems. Figure 1 illustrates the multiwell plate according to the invention, Figure 2 the positioning of the plate in the block of the thermal cycler, and Figure 3 illustrates the photograph of electrophoretically separated 454-bp fragments of human papillomavirus DNA amplified by means of rapid PCR.

One aspect of the present invention concerns the considerably decreased thickness (i.e. 10-20 fold) of the well walls when compared to conventional, thin-walled PCR plates. This can be reached, for example, rather by means of thermoforming thin thermoplastic films than by injection molding. An additional great advantage is that thermoforming due to the small tooling costs is much less expensive than high-precision injection molding which is needed to produce extremely thin parts. Such thermoplastic films are, for example, polyolefin films, such as metallocene-catalyzed polyolefin films, copolymer films and cast polypropylene films, such films having a thickness of not more than 50 microns. Preferentially, the multiwell plate is vacuumformed out of a 30-50 micron cast, unoriented polypropylene film. Usually, the film is formed into a "female" mold comprising a plurality of spaced-apart, conically shaped wells which are machined in the body of a rectangular- or square-array shaped mold. The advantage of vacuumforming into a "female" mold is that the thickness of the walls of the formed wells is gradually reduced to 15-20 µm at the bottoms of the wells. The plastic material polypropylene is compatible with the standard PCR procedures and therefore widely used for injection molding of PCR tubes and/or multiwell plates. In addition, it has a reduced water vapor sorption when compared to other plastics (e.g. polycarbonate). The volume of the wells is not more than 40 µl, preferably 16 µl, the height of the wells is not more than 3.5 mm, and the inter-well spacing is not more than 4.5 mm. Usually the number of wells is in the range of 24-96. As shown in Figure 1, the handling of the plate (1) containing the multiple wells (2) is facilitated, by a rigid 0.5-1 mm thick plastic frame (3) which is heat bonded to the plate. As shown in Figure 2, the frame (3) is not in direct thermal contact with the block (4) during thermocycling because the inner contour (5) of the frame (3) matches the outer contour (6) of the heat block (4) of the thermocycler (4, 7 and 8; 4 = block, 7 = thermoelectric heat pump and 8 = heat sink). The thickness of the well walls of the film-formed plate is reduced 10-20 fold when compared to the conventionally injection-molded PCR plates. By means of the well known Fourier equation for the heat transfer in solid substances, it can be shown that the heat transfer through the walls of the film-formed plates is 10-20 fold faster when compared to conventional PCR plates.

Another aspect of the invention is that, in order to ensure the efficient and reproducible sealing of small samples (9) by using the conventional heated-lid (10-12; 10 = heated lid, 11 = heating element, 12 = screw) technology, the conically shaped wells (2) are of identical height with similarly shaped wells machined in the body of the heat block of the thermocycler. Thus, as shown in Figure 2, the geometry of the wells enables the positioning of the entire multiwell plate (1) into the heat block (4), i.e. the parts of the multiwell plate project above the top surface of the block. In this case the pressure caused by the heated lid to the conventionally film- or silicon mat-sealed (13: seal) multiwell plate is actually directed to those parts of the multiwell plate which are supported by the top surface of the heat block (4) and not to the thin walls of the plate as it is the case for the PCR tubes or conventional PCR plates. This advantage makes it possibe to increase the sealing pressure of the heated lid several fold when compared to the conventionally used pressure of 30-50 g per well without cracking the conically shaped walls. The tight thermal contact between the extremely thin walls of the wells and the body of the block (4) is achieved automatically by increased air pressure arrising in the sealed wells at elevated temperatures. Surprisingly, by the above means of sealing the plates, samples of a volume of as few as 0.5 µl can be easily amplified without reducing the PCR efficiency. The following example serves to illustrate the invention but should not be construed as a limitation thereof.

Example:

The reaction mixture prepared according to Ting and Manos (PCR protocols, chapter 42 (1990) Eds.: Innes, Gelfand, Sninsky and White, ISBN 0-12-372180-6) and containing 10^4à input DNA copies of human papilloma virus (HPV-18), integrated into the genome of human HeLa cells, was pipetted (3-µl volume) into the wells of a 36-well ultrathin-walled plate vacuumformed out of a 47-micron thick cast polypropylene film. The samples were sealed by means of a commercial sealing film, and temperature cycled using a conventional Peltier-driven heat-block thermal cycler (ramping rate 4.5° C/second). Incubation times were as follows: Denaturing: 3 seconds at 95°C, annealing time 3 seconds at 55°C, extension time 16 seconds at 72°C, number of cycles: 30; total amplification time 20 minutes.
A photograph in Figure 3 demonstrates some results of the amplification of 454-bp long viral DNA fragments (Line 1-5: viral

DNA and line 6: molecular weight marker [Lambda-phage DNA, pstI-restriction digest]). As it can be seen from figure 3, the product yield and specificity of the exponential DNA amplification reaction was very high, although the total amplification time was 20 minutes only and the reaction was performed in the excess of human genomic DNA.

Claims

1. Ultrathin-walled multiwell plate for heat block thermocycling of samples comprising an array of small-volume wells of identical height with the similarly shaped sample wells formed in the top surface of the heat block of the thermocycler.

2. Ultrathin-walled multiwell plate according to claim 1, wherein the said multiwell plate is preferentially vacuumformed out of thermoplastic film.

3. Ultrathin-walled multiwell plate according to claim 2, wherein the said thermoplastic film is a thermoplastic film of a thickness of not more than 50 microns.

4. Ultrathin-walled multiwell plate according to claim 1, wherein the said microwell plate comprises a rigid outer frame.

5. Ultrathin-walled multiwell plate according to claim 2, wherein the said thermoplastic film is a polyolefin film.

6. Ultrathin-walled multiwell plate according to claim 5, wherein the said polyolefin film is a metallocene-catalyzed polyolefin film.

7. Ultrathin-walled multiwell plate according to claim 2, wherein the said thermoplastic film is a copolymer film

8. Ultrathin-walled multiwell plate according to claim 2, wherein the thin thermoplastic film is a cast polypropylene film.

9. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 40 µl.

10. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 20 µl.

11. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 10 µl.

12. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 5 µl.

13. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 2.5 µl.

14. Ultrathin-walled multiwell plate according to claim 1, wherein the volume of the well is not more than 1.5 µl.

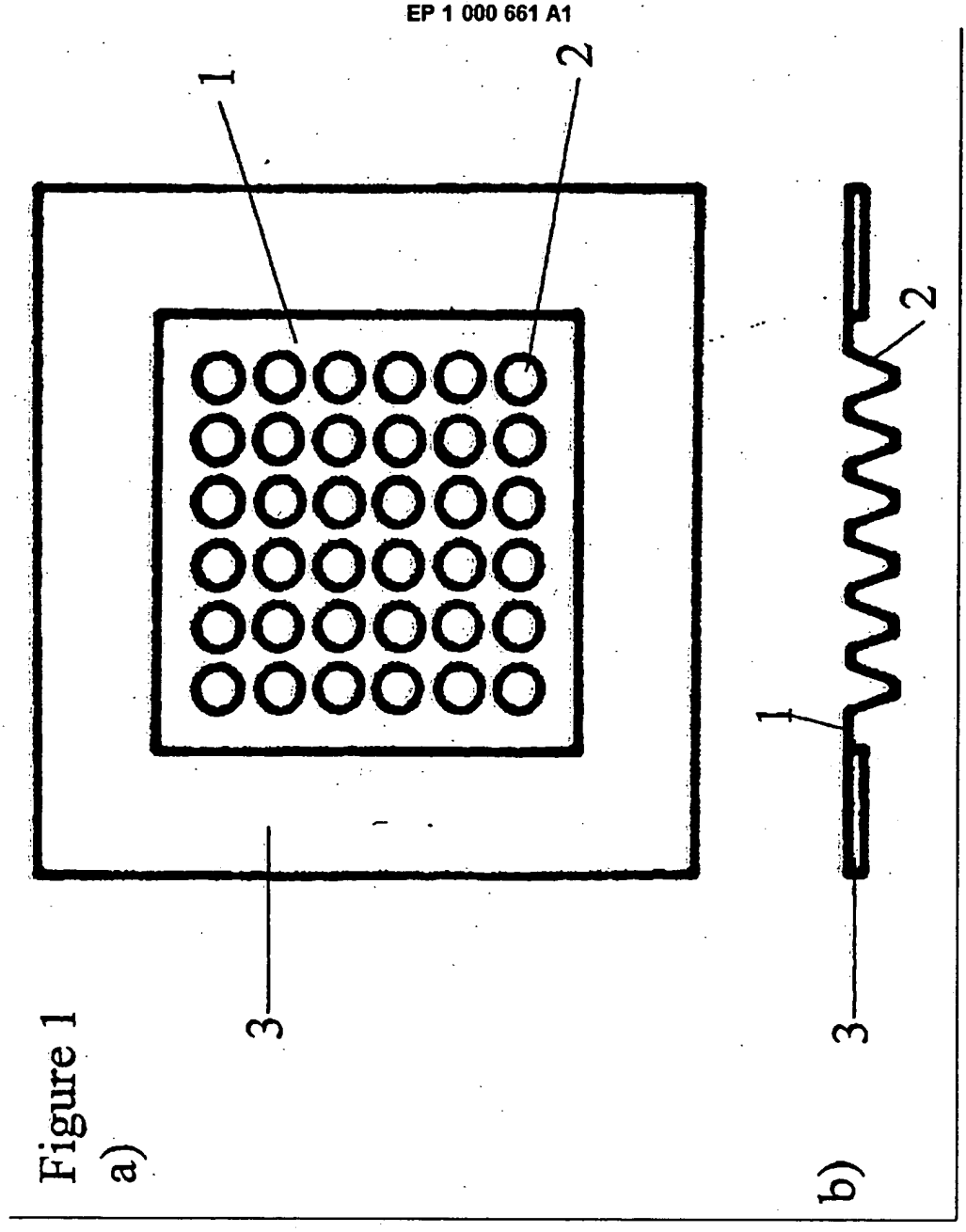


Figure 1

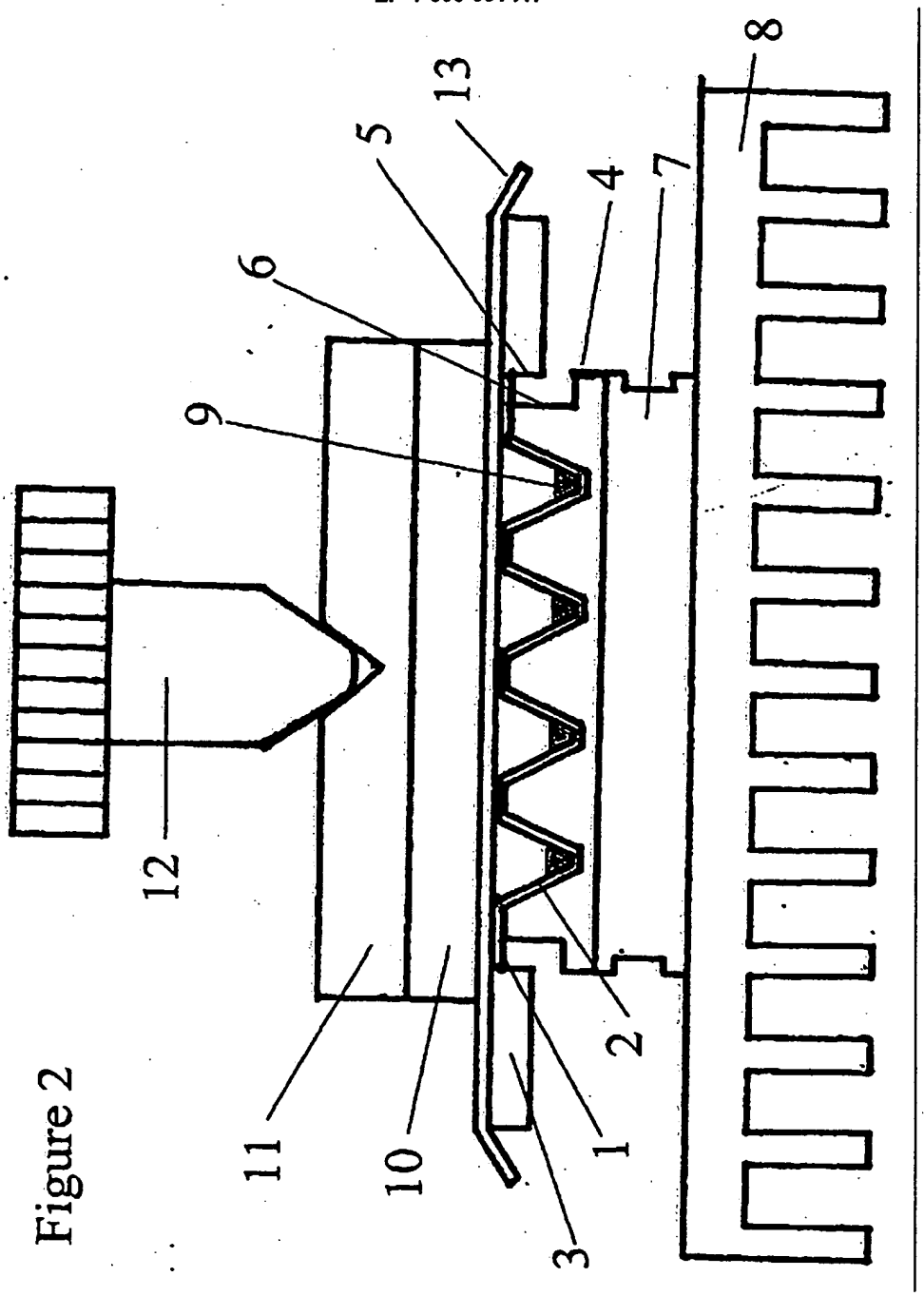


Figure 2

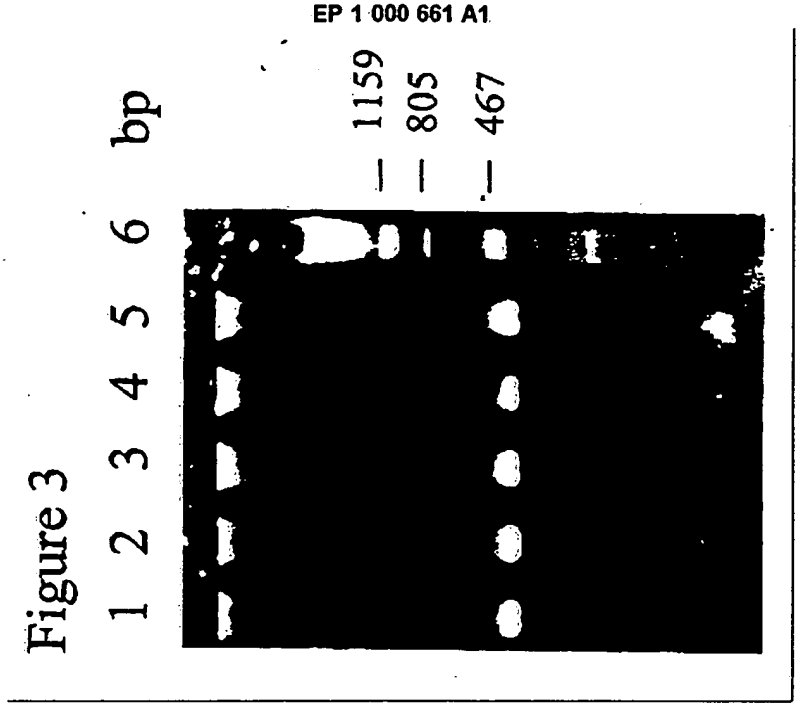


Figure 3

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Application Number
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	20 March 2000	Koch, A.

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